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Temperature affected the formation of arbuscular mycorrhizas and ectomycorrhizas in *Populus angustifolia* seedlings more than a mild drought

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ABSTRACT

Arbuscular mycorrhizal (AM) plants and fungi associate with lower soil organic matter, higher pH, lower phosphorus and higher nitrogen than ectomycorrhizal (EM) ones. However, soil conditions correlate with climatic factors, and we suggest that temperature and humidity have also direct roles in the success of mycorrhiza types. The hypothesis here is that EM perform better at low temperatures than AM, and AM resist drought better than EM.

Narrowleaf cottonwood (*Populus angustifolia* E. James) forms both AM and EM. We grew seedlings in soil at 14, 20 and 26 °C in factorial combinations with adequate watering and a cyclic mild drought for 4 and 7 weeks.

As hypothesized, the percent of EM root tips was largest at 14 °C, while the proportional root length with AM was largest at the two higher temperatures. However, unlike expectations, drought increased EM formation slightly, while the AM colonization was lower in the dry treatment. Plant growth was reduced more by low temperature than drought. Root branching was more prominent at low temperature and root length and mass growth at higher temperatures.

Soil nutrient availability did not provide a direct explanation to the results, as both soluble soil N and P were the same in 14 and 20 °C, while the change in mycorrhiza colonization took place between these temperatures. Differences in root morphology (root branching vs length) may affect the proportions of the mycorrhiza types at different temperature regimes. The most likely explanation to the differential colonization is that temperature affects AM and EM fungi in a different way. In nature, temperature and humidity regimes are tightly correlated, and temperature as such may be a stronger determinant for the success of mycorrhiza types than has been previously considered. The poorer performance of AM in low-temperature and drought conditions may reflect stress avoidance rather than stress tolerance by AM fungi.

1. Introduction

Mycorrhizas are symbioses between plants and fungi, where the plant provides photosynthates in exchange of mineral nutrients taken up by the fungus. Sometimes the relation is not mutually beneficial (Jones and Smith, 2004), but here we consider mutualistic symbioses. Arbuscular mycorrhiza (AM) is the most wide-spread type of mycorrhizas both across the plant kingdom and over different vegetation zones, while ectomycorrhizas (EM) are most common in woody plants in the cool regions of the world. There has been increasing interest in comparing arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) mutualisms in recent decades. The ability to utilize different forms of nitrogen (N) and phosphorus (P) has been considered to be a critical determinant for the success of these mycorrhiza types (Read, 1991; Read and Pérez-Moreno, 2003). The transformations of N and P are complex, affected by plant and litter properties, soil biota and climatic factors, and these cannot all be disentangled in the field. We have suggested that temperature and humidity affect the success of mycorrhiza types with their hosts more directly than has been previously assumed. The overall hypothesis is that AM are favoured by higher temperatures than EM; and that EM are favoured by ample (but not excessive) water, while AM are more drought resistant (Lehto and Zwiazek, 2011).

There is evidence for low AM colonization in cold environments from many types of studies, starting from the occurrence of host plants in

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growth chamber (Conviron GR77, Controlled Environments, Winnipeg, MB, Canada) under fluorescent tubes (VHO 215 W, Sylvania Cool White, Sylvania, USA) in 90% relative humidity, 16-h day at 20 °C, 8-h night at 16 °C. Cooling/warming rate was 5 °C h^{-1}.

We collected soil from three sites in and near Joensuu, Finland, to be used as a substrate and simultaneously as a source of EMF and AMF inoculum after transplanting. In previous trials, native European aspen (*Populus tremula*) formed preferentially EM and no arbuscules (Kilpeläinen et al., 2019), but other poplar species form both types (Khasa et al., 2002). Therefore, we expected to obtain a range of compatible EM, and possibly AM, fungi by collecting soil under *Populus* trees and seedlings, and a range of AM fungi by additional sampling of meadow soil. The sites were (1) the Joensuu arboretum *Populus* plantation with European aspen, hybrid aspen (*P.* × *wettsteinii* = *tremula* × *tremuloides)*, white poplar (*P. alba*), laurel-leaf poplar (*P. laurifolia*), balsam poplar (*P. balsamifera*) and Berlin poplar (*P.* × *berolinensis* = *P. laurifolia* × *P. nigra*) with mainly grass and forb undergrowth (62°35.97′N, 29°43.3′E), (2) Joensuu city forest park, Linnunniemi, under native *Populus tremula* trees and seedlings (62°36.5′N 29°43.2′E) and (3) Havukanaho meadow (63°3.75′N, 29°52.3′E) in Koli national park with diverse grass and forb vegetation (Kilpeläinen et al., 2016). The soil was sampled to a depth 0–20 cm with a corer of 3 cm diameter in early July. The soils from the three sites were sieved (6 mm), removing larger roots and stones and mixed in the volume proportions 1: 1: 1. The homogenized soil was mixed with perlite in proportions 2 soil: 1 perlite.

The germinated seedlings were transplanted to plastic pots (soil compressed to 185 ml, pot height 80 mm, top diameter 61 mm and base diameter 48 mm) four weeks after sowing. The same batch of soil-perlite mixture was used in all pots. We allowed for a 10-day rooting period after transplanting before applying the treatments, in otherwise the same growing conditions as before, but adding light from incandescent lamps, (60 W, Oy Airam, Finland). Day/night PAR was ca. 350/0 μmol m^{-2} s^{-1}.

The experimental treatments were chosen to represent a wide range of temperatures occurring in boreal and temperate regions during the growing season, but not extremes (e.g. Kubin and Kemppainen, 1994; Repo et al., 2007, 2014), and a mild cyclic drought, which is a more usual condition than severe drought in these vegetation zones. The target soil humidity in the dry treatment (see below) was determined by measuring leaf temperature with a portable infrared thermometer with a laser sight and macro-optics (Optris LS, Optris GmbH, Berlin, Germany) daily during water withholding; an increase of ca. 2.5 °C in leaf temperature compared to well-watered controls indicated stomatal closure. At this stage, the dry treatment pots were watered to saturation. Based on pot weights, soil water content reached ca. 18% in each cycle. The treatments were factorial combinations of temperature and watering, temperature having three levels (14/10, 20/16 or 26/22 °C day/night, abbreviated as T14, T20 and T26) and the water regime having two levels (restricted and sufficient, W0 and W1).

Groups (blocks) of six seedlings were formed at random for each of the 5 origins (mother tree genotypes). The six combinations of temperature and watering treatments were allocated at random to the seedlings within each group. The numbers of plants per origin varied, being 30 for two origins, 12 for two origins and 6 for one at the first harvest, providing 15 plants per combination of temperature and watering regime at harvest 1. At harvest 2, the numbers of plants were the same, except with 6 additional plants for the origin that had 6 at H1, therefore 16 plants per treatment factor combination. Two harvests were done, at 4 (H1) and 7 weeks (H2) after start of treatments.

The temperature regimes were assigned at random to three identical growth rooms. The daytime target air humidity was set to the same vapour pressure deficit (VPD), 0.80 kPa in the temperature regimes, corresponding to relative humidities of 50, 66 and 76%. The same RH values were set for both night and day. However, controlling air humidity at 50% RH at 10 °C was not possible due to technical limitations of the growth chambers, and the VPD remained at about 0.63 kPa. Pots were weighed regularly and watered to saturation when they had reached the same target weight corresponding to the water regime at all temperature regimes. Four pots with no plants but filled with the same soil-perlite mix were placed in each growth room and watered at the same time as the well-watered seedlings. These pots were kept in the rooms until harvest 2 and used for soluble soil-nutrient determinations. During the first 3 weeks after the start of treatments plants received only water, but later all the plants received additionally 25 ml per week of a complete nutrient solution containing 40 mg N dm^{-3} and other nutrients in proportion (Riddoch et al., 1991).

At each harvest, plant height was measured to the nearest mm with a ruler. Plants were severed at the root collar and leaves and stems were dried at 40 °C to constant mass and weighed. Subsamples of the root systems from the depths of 0.5–3.0 cm and 4.0–6.5 cm were taken from each pot for mycorrhiza observation. The subsamples were cleared and thereafter stained with methyl blue (Grace and Stribley, 1991; Kilpeläinen et al., 2016). EM root tips and non-mycorrhizal root tips were counted under a stereo microscope, and the EM proportion is expressed as percent EM root tips of the total number of root tips. AM arbuscules, vesicles, hyphae and spores as percent root length were quantified with the gridline intersection method using a stereo microscope (Giovanetti and Mosse, 1980). When necessary, roots were additionally observed under a light microscope at higher magnification.

Estimates for the total number of EM root tips and root tips per plant were computed based on the dry masses of the subsamples and the remaining parts of the root systems. Total root length in the subsamples was calculated following Tennant (1975) and the specific root length (SRL, m g^{-1}) was calculated using the dry masses of the subsamples. Total root length was estimated also for the whole root system based on the dry masses of the subsamples and remaining parts, as well as the total length with AM hyphae per plant.

At harvest 2, the soil in each of the 12 pots with no plants was dried at 40 °C. Ten grams of the soil was mixed with 100 ml 1 M KCl, shaken for 1 h and filtered (filter paper Schleicher & Schuell 589/1). NO_3–N and NH_4–N concentrations were determined from the samples by flow injection analyzer (FIAstar 5012, Tecator, Sweden). Other nutrients were analyzed with ICP-OES (Iris Intrepid II XSP, Thermo Elemental, Franklin, MA, USA) after ammonium acetate extraction in pH 4.65 (Halonen et al., 1983). At this harvest, all leaves from pairs of plants of the same origin within each treatment were pooled to have large enough samples for nutrient analyses (n = 8 per temperature and watering treatment combination). Dried leaves were ground to powder with a mortar. Nitrogen was determined with an element analyser (Varian). For the other nutrients, subsamples were digested in HNO_3 and H_2O_2 in Teflon containers (method based on Epa 3051 in microwave oven (MARS5). The nutrient concentrations were determined with the ICP-OES. Technical replicates were used to check the consistency of the analysis results. Foliar nutrient contents (total amount of a nutrient in the foliage of a seedling) were computed by multiplying the concentration by the mean dry mass of the leaves of the two seedlings in each pooled sample.

A randomized complete block design was used. Origin (O) was treated in the analysis as a block factor and harvest (H), temperature (T) and watering (W) as experimental factors. Origin had five levels, harvest two levels (H1 and H2), temperature three levels (T14, T20 and T26) and watering two levels (W0 and W1). Factorial ANOVA was used, and results discussed using $P < 0.05$ as threshold. In the case of significant interactions with harvest, the effects of temperature and watering were tested separately within each harvest. When a significant interaction was detected between temperature and watering, each watering regime was analyzed separately. Tukey's test was used to test effects of temperature levels when ANOVA indicated significance of this main effect.

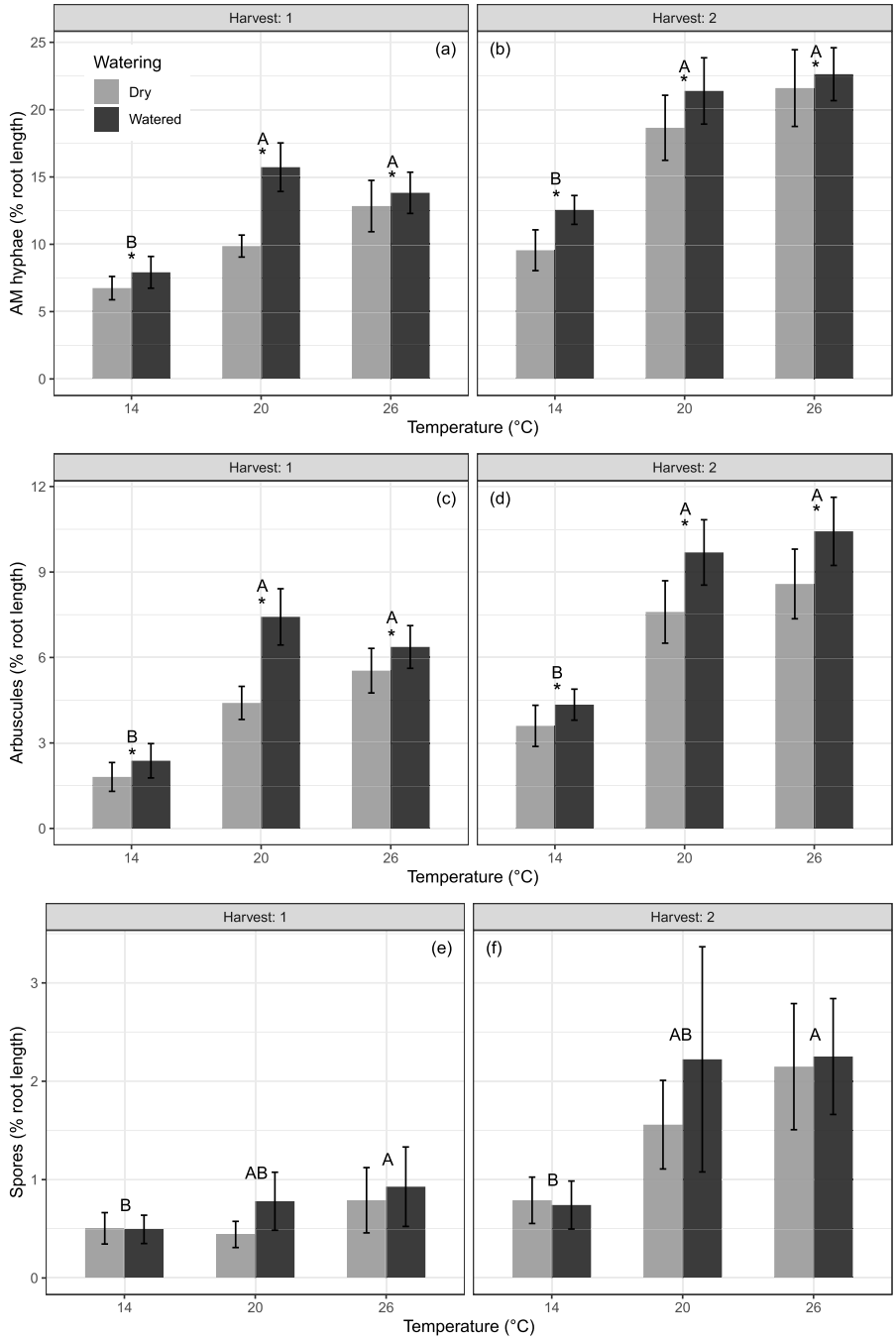

Fig. 2. Percent of root length with AM hyphae (a), (b), arbuscules (c), (d) and AM spores (e), (f) in *Populus angustifolia* at two harvests in three temperature and two water regimes (n = 15–16, means ± SE). The harvest effect was significant in each case. Means for a temperature treatment with the same letter do not differ (Tukey's test, $P < 0.05$). A significant difference ($P < 0.05$) between water regimes is shown by asterisk.

significant, but within T20 and T26 effect $P_W < 0.001$.

The plants more than doubled their dry mass between the harvests ($P_H < 0.001$; Fig. 6c and d). Plant dry mass was generally more reduced by low temperature ($P_T < 0.001$) than by the dry treatment ($P_W = 0.003$); the difference between T14 and T26 at H2 was about three-fold, while the difference between W and D at its largest, at H2 in T26 was 20% (Fig. 6). As the interaction H × T was significant ($P_{H\times T} < 0.001$), the dry mass was tested at each harvest. At both harvests, the main effects of temperature were significant (both $P_T < 0.001$), and the result of Tukey's test was the same, indicating that all temperature treatments differed; hence the interaction was caused by the larger treatment differences at H2. When the ANOVA was repeated using a ln transformation, the interaction was not significant, which confirms the conclusion.

The relative masses of leaves, stems and roots did not differ among harvests and watering regimes (main effects), but the temperature affected them ($P_T \leq 0.006$ for leaf, root and stem) (Fig. 7a and b). The stem mass ratio (SMR) was largest at T14 while T20 and T26 did not differ from each other. The interaction H × T was significant for leaf and root ($P \leq 0.001$), and the harvests were tested separately. At H1, the highest temperature treatment had more leaves and less roots than others, and T20 was intermediate ($P_T \leq 0.001$).

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